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Research Article

## Pharmacognostic, Physicochemical, Phytochemical evaluation and Thin layer chromatography of *Ocimum basilicum* leaves

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### Abstract

*O. basilicum* is a annual herb commonly referred to sweet basil and used in various traditional systems, is a member of the Lamiaceae family. The objective of this study is to gain comprehensive pharmacognostic, physicochemical characteristics, and phytochemical analysis of the leaves. The pharmacognostic criteria were assessed, including macroscopic and microscopic evaluations, transverse sections of the leaf, powder microscopy, fluorescence analysis, and physicochemical properties (Total Ash 14.60%, acid-insoluble ash 4.15%), water-soluble ash 3.27%; alcohol-soluble extractive value 2.4%; water-soluble extractive value 1.6%; moisture content 13%. Phytochemical screening of ethanolic leaf extracts indicates the presence of flavonoids, saponins, phenolic, tannins, and cardiac glycosides. The R<sub>f</sub> values determined were 0.60, 0.68, and 0.72. The standardized parameters for pharmacognostics, physicochemical parameters, phytochemical properties, total phenolic content, total flavonoids content, and chromatographic analyses of *O. basilicum* leaves are revealed in this work.

**Keywords:** Fluorescence analysis, Physicochemical, Thin layer chromatography, TPC, TFC

## INTRODUCTION

*Ocimum* genus has around 50-150 types of plants<sup>1</sup> and belonging to the Lamiaceae family. Tropical regions are really places to find *Ocimum* species. These places include Asia, Africa and India.<sup>2</sup> The *Ocimum* species are found in these areas. Sweet basil, or *Ocimum basilicum*, is a plant of the genus *Ocimum* that is found in South America, Africa, and Asia.<sup>3</sup> *O. basilicum* has large green leaves, glabrous, woody stems, and a broad, elliptical shape that measures 2.5 to 5 cm × 1 to 2.5 cm. It grows to a height of 20 to 80 cm. The 3 mm red, pink, or white flowers are grouped in terminal spikes.<sup>4</sup> In the past, *O. basilicum* fruit was used as traditional medicine to treat eye conditions, diarrhea, worm infestation, and inflammation.<sup>5</sup> In addition to being used as a tonic and vermifuge, leaves and blossoms can be used as a tea to cure diarrhea, gas, and nausea. *O. basilicum* has essential oils that are frequently used to cure wasp stings, snakebites, colds, and convulsions.<sup>6</sup> *O. basilicum* was utilized in Mediterranean cuisine, including Greek and Italian, throughout South Europe.<sup>7</sup>

## MATERIALS AND METHODS

**Plant collection:** Fresh Plant leaves of *O. basilicum* were collected from local area of Prayagraj, U.P., India. The plant material identified and authenticated by Botanical Survey of India (BSI), Prayagraj, Authentication No.11/81/2025-26/ 54/Tech/912 on dated 30/03/2026.

**Macroscopic studies:** Organoleptic characteristics, such as color, taste, texture, shape, and size, were used in morphological investigations and assessed botanically.<sup>8,9,10</sup>

**Microscopic studies:** <sup>8,9,10</sup>

Leaf was cut into tiny transverse slices for microscopic examination. For additional observations and the identification of particular microscopic diagnostic features, the slices were placed in a glycerine water solution. Additionally, the powder properties and fluorescence examination were performed.

**Physicochemical parameters**<sup>11,12</sup>

After powdering the dried plant leaves of *O. basilicum*, several physicochemical analyses were performed and values were noted, including those for foreign matter, moisture content, extractive value, and ash value.

**Phytochemical screening**<sup>13,14,15</sup>

100g of *O. basilicum* powder in a Soxhlet extractor was extracted using 95% ethanol. Following the concentration of the liquid extract, a number of qualitative chemical assays were performed and documented.

**Total Flavonoids Content**<sup>16</sup>

First, fill a graduated test tube with 50 ml of the extract, add 1 ml of methanol (Sigma Aldrich, CAS no. 67-56-1), and thoroughly mix. Next, add 4 ml of distilled water and 5% sodium nitrite (CDH, CAS no. 7632-00-0), and incubate for 5 minutes. Let it stand for ten minutes after adding the 10% w/v aluminum chloride solution (CDH, CAS no. 7446-70-0). Next, add 2 ml of 1M sodium hydroxide (Ranchem, CAS no. 1310-73-2) and use distilled water to get the content to 10 ml. A UV visible spectrophotometer is used to measure absorbance at 765 nm. The standard quercetin (Research Lab fine Chem, CAS no. 849061-97-8) absorbance is determined using the same approach. The calibration curve was used to calculate the total flavonoids content.

**Total Phenolic Content**<sup>16</sup>

Total phenolic content has been determined by FC method. One milligram per milliliter of the extract was put in a test tube; three milliliters of water was put in; then 0.5 milliliters of Folin-Ciocalteu reagent (CDH, batch no. 611221) was mixed. Then, added 20% sodium carbonate (Thomas Baker, CAS no. 497-19-8) and left it in the dark for about 10 minutes. Finally, measured the

absorbance at 650 nm with a UV-visible spectrophotometer. Likewise, measured the absorbance of gallic acid (CDH, CAS no. 5995-86-8).

**Thin layer chromatography**<sup>17,18</sup>

Using an activated silica gel G plate as the stationary phase and chloroform: Methanol: Acetic acid (6:3:1) used as mobile phase, and an iodine solution or chamber as the detecting reagent, an alcoholic extract of *O. basilicum* was assessed for TLC.

**RESULTS AND DISCUSSION**

**Macroscopic study:** The organoleptic & macroscopic characters of the fruits as colour, odour, taste, shape, size, & surface were evaluated botanically.



**Figure 1:** *Ocimum basilicum* plant

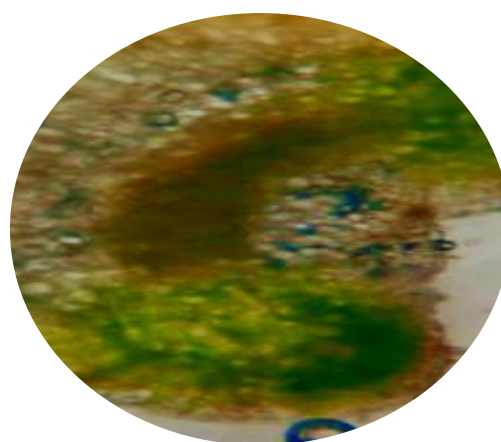
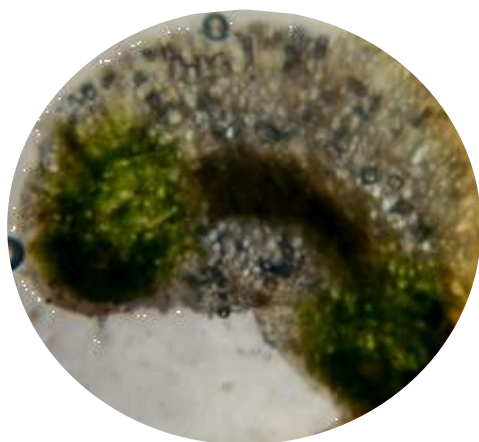
**Table 1:** Macroscopic study of *Ocimum basilicum* leaf

| Characters | Leaf                                                                    |  |  |
|------------|-------------------------------------------------------------------------|--|--|
| Colour     | Upper surface darkish green upper surface and light green lower surface |  |  |
| Odour      | Pleasant, aromatic, strong                                              |  |  |
| Taste      | Sweet                                                                   |  |  |
| Size       | 2.5-5.3 cm long, 1.5- 2 cm width                                        |  |  |
| Shape      | Simple, ovate, oval                                                     |  |  |
| Margin     | Entire, smooth                                                          |  |  |
| Apex       | Acute                                                                   |  |  |
| Venation   | Pinnate, Reticulate                                                     |  |  |
| Surface    | Pubescent                                                               |  |  |
| Base       | Attenuate                                                               |  |  |

**Microscopic study:**

Transverse section of *O. basilicum* leaf shows single layered cylindrical palisade cells; 4-6 layers of

parenchymal cells, vascular bundles were ringed by parenchymatous cells. Glandular trichomes and diacytic stomata were also present



**Figure 2:** Transverse Section of *Ocimum basilicum*

**Table 2:** Powder characteristics of *Ocimum basilicum* leaf

| S. no. | Features | Observation     |
|--------|----------|-----------------|
|        |          | Leaf            |
| 1      | Nature   | Coarse powder   |
| 2      | Colour   | Greenish        |
| 3      | Odour    | Noxious         |
| 4      | Taste    | Slightly bitter |

**Powder microscopy:** It is greenish coarse powder with bitter taste. The powder microscopic study reveals the

presence of trichomes, parenchymal cells, lignin, calcium oxalate crystal & volatile oil sac.

**Table 3:** Fluorescence analysis of *Ocimum basilicum* leaf powder

| Treatment                                    | Visible        | Long U.V. 254 nm | Short U.V. 365 nm |
|----------------------------------------------|----------------|------------------|-------------------|
| Powder                                       | Brown          | Black            | Green             |
| Powder + % KOH                               | Dark brown     | Dark             | Light green       |
| Powder + 5%NaOH                              | Blackish green | Blackish         | Green             |
| Powder + FeCl <sub>3</sub>                   | Black          | dark             | Blackish green    |
| Powder + con. H <sub>2</sub> SO <sub>4</sub> | blackish       | Dark             | Blackish green    |
| Powder + dil. H <sub>2</sub> SO <sub>4</sub> | Brown          | Dark             | Green             |
| Powder + con. HCl                            | Brown          | Black            | Dark Green        |
| Powder + dil. HCl                            | Brown          | Dark             | Greenish          |
| Powder + con. HNO <sub>3</sub>               | Black          | Dark             | Light Green       |
| Powder + dil. HNO <sub>3</sub>               | Brown          | black            | Light green       |
| Powder + dil. NH <sub>3</sub>                | Black brown    | Dark             | Green             |
| Powder + Iodine sol <sup>n</sup>             | Black -brown   | Black            | Blackish green    |

**Table 4:** Physicochemical data of *Ocimum basilicum* leaf

| S.N. | Physicochemical Parameter  | Values (% w/w) |
|------|----------------------------|----------------|
| 1.   | Foreign matter             | Nil            |
| 2.   | Moisture Content           | 13%            |
| 3.   | Total Ash                  | 14.60%         |
| 4.   | Acid- Insoluble ash        | 4.15%          |
| 5.   | Water soluble ash          | 3.27%          |
| 6.   | Alcohol soluble extractive | 2.4%           |
| 7.   | Water soluble extractive   | 1.6%           |

**Percentage yield and physical characteristics of various extract of *O. basilicum* leaf**

| Solvent extract | %w/w  | Consistency | Fluorescence analysis |            |           |
|-----------------|-------|-------------|-----------------------|------------|-----------|
|                 |       |             | Visible               | Long U.V.  | Short U.V |
| Ethanol (95%)   | 28.38 | Sticky      | Blackish brown        | Dark black | Greenish  |

**Qualitative phytochemicals screening**

Phytochemical screening of ethanolic extract of *O. basilicum* shows the presence of several secondary

metabolites. Shinoda and zinc-hydrochloric acid tests shows the presence for flavonoids while triterpenoids & alkaloids were absent. Saponins, tannins, and steroids were also present.

**Table 5:** Qualitative Phytochemical screening of Ethanolic extract of *O. basilicum*

|    | Phytochemical test                                                                                       | Ethanolic extract of <i>O. basilicum</i> |
|----|----------------------------------------------------------------------------------------------------------|------------------------------------------|
| 1. | Carbohydrates<br>i) Molisch<br>ii) Fehling Reagent                                                       | -ve<br>-ve                               |
| 2. | Alkaloids<br>i) Dragondroff's reagent<br>ii) Mayer's reagent<br>iii) Wagner reagent<br>iv) Hager reagent | -<br>-<br>-<br>-                         |
| 3. | Tannins<br>i) Lead acetate<br>ii) FeCl <sub>3</sub>                                                      | +<br>+                                   |
| 4. | Flavonoids<br>i) Shinoda test<br>ii) Zinc- HCl reduction test<br>iii) Alkaline reagent test              | ++<br>++<br>++                           |
| 5. | Saponins<br>i) Foam test                                                                                 | +                                        |
| 6. | Steroids<br>i) Libermann – Burchard test                                                                 | ++                                       |
| 7. | Cardiac glycosides<br>Keller-Kiliani                                                                     | +                                        |
| 8. | Triterpens<br>Salkowaski's test                                                                          | -                                        |

+++ More amount, ++ moderate amount, + less amount, - absent

**Table 6:** Total Phenolic Content and Total Flavonoid content

| Extract                                    | Total Phenolic content mg GAE/g | Total Flavonoid content mg Quercetin equivalent/g |
|--------------------------------------------|---------------------------------|---------------------------------------------------|
| Ethanol extract <i>O. basilicum</i> leaves | 25.40                           | 18.30                                             |

**Table 7:** TLC Profile: Thin layer chromatography of alcoholic extract of *O. basilicum*

| Solvent system                            | No. of spots | Rf value         |                                                                                     |
|-------------------------------------------|--------------|------------------|-------------------------------------------------------------------------------------|
| Chloroform: Methanol: Acetic acid (6:3:1) | 3            | 0.60, 0.68, 0.72 |  |
| Spraying agent/Detection : Iodine chamber |              |                  |                                                                                     |

## DISCUSSION:

T.S. of *O. basilicum* shows isodiametric epicarp with thin cuticle, mesocarp have 5-7 layers of large parenchymatous elongated cells with starch grains, vascular bundles are present in the mesocarp. Powder microscopy reveals the presence of parenchymal cells, trichomes, & calcium oxalate crystals. Qualitative phytochemical screening indicates presence of alkaloids, tannins, triterpenes, steroids and flavonoids, carbohydrates; total phenolic content and total flavonoids contents were found 25.40 mg GAE/g and 18.30mg Quercetin eq/g respectively; thin layer chromatography of ethanolic extracts indicates presence of many compounds.

## CONCLUSION:

The plant *O. basilicum* is a common species that has been used traditionally. The above pharmacognostic, physicochemical, phytochemical & chromatographic studies will give approaches for identification, safety & quality parameters as well as new incentive to natural system of medicine in the research & in the treatment of other diseases.

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